THESIS

EFFECTS OF OZONE ON SEEDLINGS OF ROCKY MOUNTAIN PONDEROSA PINE

Prepared by

William Mark Aitken

Botany and Plant Pathology Department

In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall, 1982

LIBRARY COPY
ROCKY MT. FOREST & RANGE
EXPERIMENT STATION

Thesis/
Report
Aitken
[redacted]
J. M.

COLORADO STATE UNIVERSITY

FALL, 1982

WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR SUPERVISION BY WILLIAM MARK AITKEN ENTITLED EFFECTS OF OZONE ON SEEDLINGS OF ROCKY MOUNTAIN PONDEROSA PINE BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE .

Committee on Graduate Work

Adviser

Department Head

ABSTRACT OF THESIS

EFFECTS OF OZONE ON SEEDLINGS OF ROCKY MOUNTAIN PONDEROSA PINE

The large metropolitan areas of Colorado are recognized as locations with potential photochemical oxidant air pollution problems. Photochemical oxidant injury causes a severe chlorotic decline to ponderosa pine (*Pinus ponderosa* var. *ponderosa*) in the southern California mountains. Ozone is the principal cause of the chlorotic decline. To determine whether the Colorado variety of ponderosa pine is sensitive to ozone, two-year-old Rocky Mountain ponderosa pine seedlings (*Pinus ponderosa* var. *scopulorum*) were fumigated in greenhouse fumigation chambers at 0.50, 0.40, 0.30, or 0.25 ppm for 21 days, 10 hr/day from 8:00 A.M. to 6:00 P.M. Seedlings fumigated at 0.50, 0.40, and 0.30 ppm exhibited symptoms of needle mottle and chlorosis when compared to control chamber seedlings. No visual symptoms were observed on the seedlings fumigated at 0.25 ppm. Four-year-old seedlings fumigated at 0.50 ppm ozone for 18 days, 9 hr/day, had significantly lower needle chlorophyll contents for all four needle ages, confirming visual observations. This evidence suggests that Rocky Mountain ponderosa pine is sensitive to ozone, with the threshold ozone concentration for visual injury lying between 0.25 and 0.30 ppm. Thus, the potential exists for oxidant injury to ponderosa pine stands. Field fumigations and an expanded monitoring program would

be necessary to adequately determine whether ozone is damaging ponderosa pines along the Colorado Front Range.

William Mark Aitken
Botany and Plant Pathology Department
Colorado State University
Fort Collins, Colorado 80523
Fall, 1982

ACKNOWLEDGEMENTS

I gratefully acknowledge the assistance of my adviser, Dr. William R. Jacobi, for all his encouragement, support, and guidance during my graduate experience.

I thank the other members of my graduate committee: Dr. John M. Staley and Dr. C.P.P. Reid for their help in the construction of the fumigation apparatus and their valuable assistance in the preparation of this thesis.

Special thanks are extended to Lisa H. Young for her support, patience and understanding during this entire undertaking.

I would also like to thank Dr. Frank G. Hawksworth, the U.S.D.A. Forest Service, Rocky Mountain Forest and Range Experiment Station, and the Eisenhower Consortium for Western Environmental Forestry Research for providing the funding necessary to complete this project.

TABLE OF CONTENTS

Chapter		Page
I.	INTRODUCTION	1
II.	LITERATURE REVIEW	3
	Ozone Damage to California's Ponderosa Pine	7
	Physiological Effects of Ozone	9
	Histological Observations	12
	Effects of Other Pollutants	14
	Variation in Susceptibility to Ozone	16
	Effect of Tissue Age on Susceptibility	17
	Environment and Ozone Interactions	18
	Indicator Plants	20
	Impact of Air Pollution on Forest Ecosystems and Plant Populations	21
	Effects of Pollutants on Parasitic Diseases of Plants	23
	Exposure Chamber Systems	24
III.	MATERIALS AND METHODS	25
IV.	RESULTS	30
V.	DISCUSSION	39
	REFERENCES	43
	APPENDICES	50
	Appendix 1	51
	Appendix 2	52
	Appendix 3	53

Table of Contents (cont')

Page

Appendix 4 54

Appendix 5 55

Appendix 6 57

Appendix 7 59

Appendix 8 60

CHAPTER I

INTRODUCTION

The Denver Metropolitan area of Colorado has a serious air pollution problem, averaging over 150 days per year with poor or worse air quality (Colorado Department of Health, 1980b). One of the major pollutants of concern is ozone. In 1979, violations of National Ambient Air Quality Standards for ozone were recorded at 6 of the 10 sites in the Colorado Front Range where ozone is monitored (Colorado Department of Health, 1980a).

Ozone levels are sufficient in and around Denver to elicit plant response (Anderson et al., 1977; James and Staley, 1980). Studies in California have reported that the coastal variety of ponderosa pine (*Pinus ponderosa* var. *ponderosa*) is highly susceptible to oxidant air pollution injury (Miller et al., 1963; Richards et al., 1968; Williams et al., 1977). Limited work has been conducted to determine whether the Colorado variety of ponderosa pine (*Pinus ponderosa* var. *scopulorum*) is sensitive to ozone (Staley, *personal communication*).

James and Staley (1980) conducted surveys within and adjacent to the Denver Metropolitan area to locate air pollution injury on ponderosa pine. Some of the trees examined displayed typical oxidant air pollution symptoms. They concluded that controlled ozone fumigation experiments to identify injury thresholds coupled with an

expanded ozone monitoring program would be needed to study if injury observed on ponderosa pine foliage is actually caused by oxidant air pollution.

This paper reports on the relative sensitivity of Rocky Mountain ponderosa pine seedlings to controlled ozone fumigations. The ozone injury threshold to ponderosa pine seedlings was identified. Chlorophyll extractions were performed on fumigated seedlings in an attempt to confirm and quantify visual injury symptoms. Results of preliminary ambient air monitoring for oxidants are presented. Two ozone sensitive plants native to Colorado possibly useful as indicator plants were identified.

CHAPTER II

LITERATURE REVIEW

The Denver Metropolitan area of Colorado has a serious air pollution problem. For the years 1975-1977, Denver's air quality, as determined by the President's Council on Environmental Quality, put it in the worst group of Standard Metropolitan Statistical Areas, averaging over 150 days per year with poor or worse air quality (Colorado Department of Health, 1980b).

The individual pollutants of concern in the Denver area are carbon monoxide (CO), ozone (O_3), and total suspended particulates (TSP) (Colorado Department of Health, 1980b). The National Ambient Air Quality Standards (NAAQS) for these three pollutants are: (1) CO = 9 parts per million (ppm)[1] averaged over an 8-hour period, not to be exceeded more than once per year; (2) O_3 = 0.12 ppm averaged over a 1-hour period, with the statistically estimated number of days per calendar year with maximum hour average > 0.12 ppm not to be exceeded more than once; and (3) TSP = 260 micrograms per cubic meter ($\mu g/m^3$) averaged over a 24-hour period, not to be exceeded more than once per year (Colorado Department of Health, 1980a, 1980b). National Ambient Air Quality Standards have been exceeded frequently for these three pollutants since measurements in the downtown Denver area began in 1965 (Anderson et al., 1977).

---

[1] 1.0 ppm = 1960 $\mu g/m^3$.

Ozone, a photochemical oxidant, is a major phytotoxicant in crop plants, with ozone symptoms observed in a wide range of vegetation (Heggestad, 1968; Hepting, 1964; Hill et al., 1961; Neagele, 1973; Rich, 1964; Richards et al., 1965; and Threshow, 1970). Plants affected by ozone include leafy vegetables, field, forage and textile crops, also shrubs, broad-leafed ornamental, fruit and forest trees. A reference work is available examining the effects of air pollutants, individually and synergistically, on both higher and lower plants (Mudd and Kozlowski, 1975).

Photochemical oxidant pollution is a complex system with ozone the major component. Ambient ozone occurs at low concentrations (0.05 ppm) due to natural physical and chemical phenomena (Colorado Department of Health, 1980a). Ozone is not emitted directly from a source as other pollutants are, but forms as a secondary pollutant. It is produced in high concentrations in urban airsheds by chemical reactions in the atmosphere mainly between oxygen, nitrogen oxides, and gaseous hydrocarbons (Anderson et al., 1977). Nitrogen oxides are emitted mainly in automobile exhaust, but come also from gasoline and oil storage and transfer, from industrial use of paint solvents, degreasing agents, cleaning fluids, ink solvents, incompletely burned coal or wood and many other sources (Colorado Department of Health, 1980a).

Denver's climate and topography combine to create a unique pollution situation. The city has an unusually high number of temperature inversions. Denver experiences higher solar intensity levels than other metropolitan areas at lower elevations--maximum ozone concentrations have been directly correlated to solar

intensity (McCormick, 1968). The highest ozone levels generally occur in Denver during summer when the high temperatures and strong sunlight promote photochemical reactions (Anderson et al., 1977; Colorado Department of Health, 1980a). High pressure systems around Denver will create a light wind period for several successive days, increasing the chances for heavy pollution (Anderson et al., 1977; Riehl and Crow, 1962).

Topographically, Denver is an isolated city, with most pollution stemming from sources within the metropolitan area (Riehl and Crow, 1962). The pollution pattern is typical of an urban region dependent on automotive transportation. The commuting auto fleet of Denver consists of 90 cars for every 100 commuters (Anderson et al., 1977). The topographic features around Denver strongly influence wind direction. The Rockies arise west of the city, providing a typical upslope-downslope diurnal wind pattern superimposed on other effects (Anderson et al., 1977). Denver is also situated in the depression of the South Platte River, with land elevation decreasing to the northeast. During night and morning, air drains down the Platte toward northeast, often stopping in the northeast suburbs. During the afternoon, the air flow reverses, and much of the polluted air re-enters the city (Riehl and Crow, 1962; Riehl and Herkhof, 1970). This back and forth movement of air will persist as long as light winds remain dominant (Riehl and Crow, 1962). These common wind reversals may cause pollutants to reach the forested mountains west of Denver (James and Staley, 1980).

High concentrations of ozone have been known to exist in the Denver Metropolitan area. The highest level of ozone recorded in

Denver was 0.375 ppm on July 9, 1974 (Colorado Department of Health, 1980b). In 1979, violations of NAAQS for ozone were recorded at six of the ten sites in the Colorado Front Range where ozone was monitored (Colorado Department of Health, 1980a). Due to the time necessary for sunlight to work on ozone precursors, levels of ozone are frequently found in higher concentrations away from the source (Anderson et al., 1977). Ozone pollution in the Denver Metropolitan region is a problem more pronounced in suburban areas not associated with traffic activity than it is in urban areas (Colorado Department of Health, 1977).

Anderson et al. (1977) predict that peak ozone concentrations in the Denver Metropolitan area will decrease significantly in the next three decades in spite of projected population and vehicle growth if the Federal vehicle emission standards of 1985 are met. Failure to meet these standards would impede achievement of air quality standards (Anderson et al., 1977).

Ozone levels are sufficient in and around Denver to elicit plant response (James and Staley, 1980). Studies in California have reported that ponderosa pine (*Pinus ponderosa* var. *ponderosa*) is highly susceptible to oxidant air pollution injury (Miller et al., 1963; Parmeter et al., 1962; Williams et al., 1977). Limited work has been done to determine whether the Colorado variety of ponderosa pine (*Pinus ponderosa* var. *scopulorum*) is sensitive to ozone (Staley, *personal communication*). James and Staley (1980) conducted surveys within and adjacent to the Denver Metropolitan area to locate photochemical air pollution injury on Rocky Mountain ponderosa pine. Trees within the area surveyed were found exhibiting shortened needles, reduced needle complement, and chlorotic banding of the foliage. Five evaluation plots were

established to monitor oxidant injury on ponderosa pine over time. They concluded that controlled ozone fumigation experiments coupled with an expanded monitoring program would be essential to studying whether injury to ponderosa pine foliage is actually caused by oxidant air pollution.

Ozone Damage to California's Ponderosa Pine[2]

One of the first occurrences of a photochemically produced air pollutant causing tree injury began in the 1940s and 1950s in southern California (Darley, 1968). Scattered trees within ponderosa pine stands in the Los Angeles basin began to show symptoms of decline. Asher (1956) first described the symptoms of the chlorotic decline, which included needle mottle, premature senescence and needle drop. Parmeter et al. (1962) characterized the symptoms of the chlorotic decline in California as having: a progressive reduction in terminal and diameter growth; loss of all but the current season's needles; reduction in number and size of needles; yellow mottling of the needles; deterioration of the fibrous root system; and eventual death of trees. In affected ponderosa pine stands a certain percentage of the trees were observed to remain visibly unaffected while others were severely injured or dead, suggesting a wide range of variation in susceptibility (Parmeter et al., 1962).

Since no pathogenic agent could be found, the cause of the disease remained unknown and the name "X-disease" was adopted by foresters (Asher, 1956; Parmeter et al., 1962). This decline followed approximately the same pattern of development as that

---

[2]All further references to ponderosa pine are to *Pinus ponderosa* var. *ponderosa*, not *Pinus ponderosa* var. *scopulorum*, unless otherwise stated.

described by Linzon (1960, 1966) and Berry (1961) for emergence tipburn of eastern pine (*Pinus strobus* L.). Ozone, a constituent of smog, was suggested as the cause of the emergence tipburn (Berry and Ripperton, 1963). Air pollution, drought, or a combination of the two were suggested as possible causes of the chlorotic decline of ponderosa pine in the San Bernardino Mountains of southern California (Parmeter et al., 1962).

Miller et al. (1963) critically compared the needle symptoms from naturally declining ponderosa pine trees receiving an average daily peak oxidant concentration of 0.09 ppm to those symptoms produced artificially in a controlled fumigation chamber containing 0.50 ppm[3] of synthesized ozone. Visibly similar symptoms included: (1) chlorotic mottling of the needles beginning with older whorls and progressing to the current season's needles; (2) abscission of the chlorotic needles progressing from older to younger except for the current season's needles. The chlorophyll content of needles treated with ozone for 18 days was generally less than that of ambient air controls, confirming visual observations of chlorotic mottling. They concluded that atmospheric ozone may be the primary etiological factor responsible for the chlorotic decline and needle mottling of ponderosa pine in the San Bernardino Mountains. Miller and Parmeter (1965) then conducted sustained low-concentration ozone fumigations on ponderosa pine seedlings and found typical chlorotic needle symptoms after 3 to 4 weeks' exposure at 0.30 ppm ozone for 9 hours per day.

---

[3] 0.50 ppm = 980 μg/m³.

Richards et al. (1968), conducting controlled fumigation between 0.20 and 0.50 ppm ozone, determined that mottling appeared first at the needle tip in current or emerging needles, continued toward the base as the needle tissue matured, and was followed in sequence by a distinctive chlorosis and yellowing, premature senescence and needle drop, providing a reliable sequential diagnostic ozone symptom pattern. They reported 15 species of pine affected by ozone in various areas of southern California, and emphasized that needle mottle of pine was a distinctive physiogenic disease. These findings confirmed the work of Miller et al. (1963), namely that atmospheric ozone was the prime etiological factor responsible for the chlorotic decline of ponderosa pine in southern California.

Important distinctions have been found to exist between the injuries attributed to chronic and acute ozone injury to ponderosa pine (Miller and Evans, 1974; Richards et al., 1968). Chronic ozone injury is present on both abaxial and adaxial surfaces, exhibiting relatively indefinite boundaries, with small islands of green or yellow tissue often present (Miller and Evans, 1974). Acute injury may directly induce a definitive type of tip necrosis of the emerging needles not visibly preceded by, or associated with, chlorotic mottling of the type induced by a chronic exposure concentration (Richards et al., 1968). Acute injury has well-defined boundaries and all tissue is necrotic (Miller and Evans, 1974).

Physiological Effects of Ozone

Several scientists have studied the physiological effects of air pollutants on pine (Barnes, 1972a, 1972b; Miller, 1969; Tingly

et al., 1976; Wilkinson and Barnes, 1973). Miller et al. (1969) investigated the ozone dosage response of ponderosa pine seedlings. Three-year-old pines fumigated in controlled environment chambers with ozone at 0.15, 0.30 and 0.45 ppm had apparent photosynthesis rates reduced by 10, 70 and 85 percent respectively, after 30 days exposure. A fumigation with 0.30 ppm ozone for 33 days depressed the cold-perchloric acid-extracted-polysaccharides of both current and one-year-old needles by 40 percent. The 80 percent ethanol soluble sugar concentration of current year, ozone-injured needles increased 16 percent and that of the one-year-old needles decreased slightly. Both carbohydrate fractions of control trees in carbon-filtered air increased moderately. All results were significant except the 16 percent increase in sugar concentration of current year, ozone injured needles. Apparent photosynthesis rate was considered a sensitive index for ozone dosage response. The theory was advanced that depletion of needle carbohydrates probably induces premature abscission. Significant increases in total soluble carbohydrates, reducing sugars, and ascorbic acid were found in five pine species chronically exposed to 0.05 ppm ozone for 5 to 22 weeks (Barnes, 1972b), corroborating the findings of Miller et al. (1969).

Tingly et al. (1976) studied the effect of chronic ozone exposures (200 $\mu g/m^3$, 6 h/day; 7 days/wk for variable periods up to 20 weeks) on the metabolite content of ponderosa pine seedlings. Ozone treatment had no effect on the nitrogen or amino acid content of seedling tops but significantly increased the levels in the roots. They suggest the increase is possibly due to translocation from the injured tops. The levels of soluble sugars, starch, and phenols

tended to increase significantly in the tops and decrease significantly in the roots of ozone-exposed trees. They suggest that this is due to the formation of glycosides that render the metabolites unavailable for translocation to the roots or growth. Ozone fumigation had no effect on the lignin content of roots or tops.

Seedlings of four species of pine chronically exposed to 0.5 and 0.15 ppm ozone showed a depression in photosynthesis and a stimulation of respiration, with greater effects noted at higher ozone concentrations (Barnes, 1972a). Foliage of various ages responded differently to ozone exposures. Younger secondary needles were more sensitive to ozone, showing a greater depression in photosynthesis and a greater stimulation in respiration, while older needles responded unpredictably.

Coyne and Bingham (1981) studied photosynthesis and stomatal conductance of attached needles in relation to cumulative ambient ozone concentrations (0.03 ppm) on sapling ponderosa pines in the San Bernardino National Forest. The sapling needles were exposed for 0-800 hours. They found the decline in photosynthesis and stomatal function normally associated with aging was accelerated as ozone injury symptoms increased. In all trees and needle ages, losses in photosynthetic capacity exceeded reductions in stomatal conductance.

Wilkinson and Barnes (1973) reported on the effects of ozone on $^{14}CO_2$ fixation patterns in seedlings of white (*Pinus strobus* L.) and loblolly (*Pinus taeda* L.) pines. The major changes in distribution of ^{14}C were: (1) a reduction in soluble sugars; (2) an increase in sugar phosphates; and (3) an increase in free amino acids, especially alanine. These differences were observed in seedlings fumigated at

0.10 ppm ozone for 10 minutes. These results were significant, however, other combinations of ozone concentrations and exposure times did not always result in statistically significant effects.

Higher concentrations of ascorbic acid found naturally in older needles may protect plants from ozone induced injury (Freebairn, 1960). Miller et al. (1969), however, found that higher, endogenous ascorbic acid concentrations in older needles did not act as a protectant against ozone injury to ponderosa pine needles.

Histological Observations

Histological and histochemical examinations have been used to assay ozone injury in pines (Evans and Miller, 1972a, 1972b, 1975; Linzon, 1967; Miller and Evans, 1974). Evans and Miller (1972a) reported that the plicate mesophyll cells of ponderosa pine were most sensitive to ozone fumigation, and no discernible effects were found in other cell types after 35 days of fumigation at 0.45 ppm ozone for 12 hrs/day. Thus, ponderosa pine differs from eastern white pine in which cells of the endodermis and stele are injured (Linzon, 1967). No histological difference was found between the injuries caused by ozone and by total oxidants (ozone, NO_2, PAN) on needles of ponderosa pine (Miller and Evans, 1974). In both treatments, injury was confined to the mesophyll cells, beginning on the abaxial leaf portion and extending to the adaxial leaf mesophyll with extended fumigation (Evans and Miller, 1975; Miller and Evans, 1974).

After five days of fumigation at 0.45 ppm ozone, chloroplasts and a carbohydrate specific stain accumulated in the peripheral portions of ponderosa pine mesophyll cells, with subsequent cell wall folding, twisting and cell collapse (Evans and Miller, 1972a).

Concurrently, the homogeneous distribution of proteins and nucleic acids was disrupted. Histological and histochemical changes occurred within 5-7 days, but visual symptoms were not evident until 2-3 weeks after fumigation (Evans and Miller, 1972a).

Evans and Miller (1972b) evaluated the relative ozone sensitivity of four *Pinus* species according to morphological and histological characteristics. The secondary leaves of the more ozone-sensitive species, including ponderosa pine, exhibited a larger number of stomata per cross-sectional area of mesophyll cells while the number of mesophyll cells per stomata was lower. The number of hypodermal layers and thickness of epidermal and hypodermal layers were negatively correlated with ozone sensitivity. Ozone injury affected a greater proportion of the entire needles of the sensitive species as evidenced by histological symptoms closer to the needle base (Evans and Miller, 1972b).

Injury to ponderosa pine from ozone has been reported to be distinguishable visually and/or histologically from injury caused by other pollutants and winter fleck injury (Evans and Miller, 1972a, 1975; Miller and Evans, 1974; Solberg et al., 1955). Solberg et al. (1955) found chloroplast destruction, plasmolysis, and eventual collapse in the mesophyll of ponderosa pine following exposure to hydrogen fluoride. The endodermal walls were distorted after treatment, and hypertrophy and hyperplasia were found in the resin duct epithelium. Evans and Miller (1972a) found no such effects during their ozone exposures.

Evans and Miller (1975) reported that SO_2 damaged all types of ponderosa pine cells and was not confined to the plicate parenchyma as was ozone. Unlike ozone, SO_2 did not result in an aggregation of cellular constituents, and numerous intact plastids were present in

cells with extensive cell wall deformations (Evans and Miller, 1975). In addition, SO_2 caused direct visual banding on ponderosa pine, while ozone caused large irregularly shaped necrotic lesions which only occasionally coalesced to resemble a band (Evans and Miller, 1975).

Winter fleck injury of ponderosa pine, unlike ozone, was not confined to the mesophyll cells, but included endodermal, transfusion, and vascular cells (Miller and Evans, 1974). Hyperplasia in the endodermis and transfusion area was common only with winter fleck (Miller and Evans, 1974).

Effects of Other Pollutants

Ponderosa pine seedlings have been reported to be very tolerant to acute levels of PAN (peroxyacetyl nitrate) (Davis, 1975). The response of ponderosa pine primary needles to separate and simultaneous ozone and PAN exposures has been studied (Davis, 1977). The seedlings were visibly injured by ozone but not by PAN. Simultaneous exposure of 6-week-old seedlings to ozone and PAN resulted in significantly less damage than from ozone exposures alone. This reaction did not occur with 10-week-old seedlings. Davis (1977) hypothesizes that the leaf tissue must be in an ozone-sensitive stage in order for PAN to have an influencing effect, and that plant response to simultaneous PAN and ozone exposure will likely vary depending upon factors such as species, dosage, ratio of combined pollutants, age of plant or leaf, leaf surface evaluated, environmental conditions, and method of evaluation.

It has been reported that sulfur dioxide causes foliar injury to ponderosa pine at concentrations of 0.25 ppm or less (Scheffer and Hedgcock, 1955). Damage was observed as far as 50 miles from the pollutant sources. Karpen (1970) suggests a possible

synergistic effect resulting from an interaction of sulfur dioxide with naturally occurring ozone in the Puget Sound region of Washington. Histological effects of sulfur dioxide on ponderosa pine have also been reported (Evans and Miller, 1975).

Studies have shown that ponderosa pine is sensitive to fluorides. Adams et al. (1952) attributed to fluoride compounds the damage to 50 square miles of ponderosa forest at Spokane, Washington. Analysis of the air and rainfall pointed to a similar correlation between fluoride content and distance from the source. Shaw et al. (1951), from field observations, found damage to the ponderosa forests to be zoned in its intensity about a center coinciding with the industrial area north of Spokane. Chemical analysis of needles indicated that fluoride content decreased with increasing distance from the pollution source, values decreasing from 620 ppm at points one mile from the source to 25 ppm nine miles away.

Confirmation of the conclusion that damage was due to atmospheric fluoride compounds was obtained by transplanting 5-year-old seedlings of *P. ponderosa* to three sites within the area of suspected fluoride pollution (Adams et al., 1956). A linear relationship was found to exist between atmospheric fluoride content on these sites, and the damage caused to transplants. Injury similar to that observed on the field transplants appeared when ponderosa pine seedlings were fumigated for 24 hours with 12 ppb of hydrogen fluoride (Shaw et al., 1951). Adams et al. (1956) carried out fumigation experiments with hydrogen fluoride at 0.7, 1.2, and 12.0 ppb for 24, 48, 72, 96 and 120 hours, using 5-year-old ponderosa seedlings. They concluded that needles were many times more sensitive to damage when in the immature

partially elongated state, than when mature. Walter (1956) found considerable variation among even-aged ponderosa pine seedlings in their sensitivity to atmospheric fluoride.

Variation in Susceptibility to Ozone

Inherent variation in susceptibility to atmospheric ozone has been reported for ponderosa pine (Miller 1973a; Parmeter et al., 1962; Richards et al., 1968), eastern white pine (Berry, 1961; Berry and Ripperton, 1963; Hepting, 1966; Linzon, 1966) and many other pines found susceptible to ozone (Davis and Wood, 1972; Taylor and Richards, 1959-60). Davis and Wood (1972), in studying the relative susceptibility of eighteen coniferous species to ozone, found in sensitive species a tremendous amount of variation in symptom development from tree to tree within a given species, from branch to branch on a given tree, and from needle to needle within the same fascicle. Needles on terminal branches were generally more sensitive than were needles on side branches, current foliage was more sensitive than older foliage, and plants in dormant condition were generally resistant. Richards et al. (1968) makes the assumption that such variations occur in all pine species susceptible to ozone, and concludes that the ultimate solution to ozone injury in certain species may be selection or breeding for resistance.

Miller (1973a) reported that ponderosa pine 1-year-old needles were injured three times as severely as were current year needles. Parmeter et al. (1962) emphasized that in all affected ponderosa pine stands he studied, a certain percentage of trees had been observed to remain visibly unaffected while others were

severely injured or dead. Richards et al. (1968) reported that some affected ponderosa pine trees in California may retain only current-year needles while others may show retention of affected needles for two, three, and four seasons.

Effect of Tissue Age on Susceptibility

Several researchers have reported on the influence of plant age on the sensitivity of pines to ozone (Berry, 1974; Davis, 1977; Davis and Coppolino, 1974; Davis and Wood, 1973b). Davis and Wood (1973b) reported that all three Virginia pine needle types (cotyledon, primary, and secondary) reached maximum sensitivity to ozone at approximately 3 to 5 weeks following initiation of needle growth, after which time the sensitivity declined. Cotyledons became resistant at 16 weeks; primary needles were still sensitive at 16 weeks, the oldest age studied; secondary needles became resistant at 18 weeks. The basal portions of the cotyledons were more sensitive than the tip or mid portions. The youngest primary and secondary needles were injured most severely at the needle tip, whereas older needles were more severely injured at the base.

Berry (1974) tested four species of southern pine and found that the primary needles of the seedlings tested were most sensitive to ozone at 2 weeks of age, or possibly earlier. In contrast, Davis (1977) found that the period of maximum ozone sensitivity of ponderosa pine primary needles occurred within 3-8 weeks of age, with sensitivity increasing rapidly from 1 to 4 weeks and then declining more gradually, until the needles became quite tolerant of ozone at approximately 12 weeks of age.

Davis and Coppolino (1974) studied the relationship between age and ozone sensitivity of current needles of 2-year-old ponderosa pine seedlings and reported that current needles exhibited maximum sensitivity to ozone during the third to ninth week of age. Immature needles were generally injured most severely at the tips; injury on mature needles was more general. Miller (1973a) found that 1-year-old ponderosa pine needles were three times as sensitive as the current needles to chronic ozone exposures and were most sensitive to exposures begun in mid-August. In contrast, Davis and Coppolino (1974) observed these needles to be fairly resistant to acute doses of ozone, and that the current needles of various conifer species subjected to acute doses of ozone were most sensitive in late spring to early summer. Differences in these reports may be due to inherent differences in experimental design, or to differing environmental conditions during exposure of plants to ozone (Davis and Coppolino, 1974).

Environment and Ozone Interactions

Many studies have been written concerning factors which affect plant sensitivity to air pollutants (Berry, 1974; Costonis and Sinclair, 1969; Davis, 1977; Davis and Coppolino, 1974; Davis and Wood, 1973a, 1973b; Heck et al., 1965). Heck et al. (1965) present a review of the work relating plant injury to specific air pollutants as conditioned by several environmental conditions, including light (quality, intensity and duration), temperature, humidity, soil moisture, and nutrient levels. Research supports the contention that the stomatal apparatus must be open before the phytotoxicants can enter the sensitive mesophyll areas of the leaf and cause damage, and that the stomates are only a pathway for entrance of the pollutant, and not the primary

mechanism controlling injury. The conclusions they reported were: (1) plants grown under an eight-hour photoperiod are more sensitive than those grown in a longer photoperiod; (2) sensitivity during exposure is directly correlated with light intensity. A minimum intensity is necessary for injury, and injury increases to a maximum with increasing sensitivity; (3) plants grown at low temperatures for one to several days before exposure are less sensitive than those grown at higher temperatures; (4) plants are more sensitive to higher temperatures at time of exposure, but this might be a compounded light effect and higher temperatures may actually result in reduced sensitivity; (5) plants grown under semi-drought conditions are less sensitive than those with adequate water; (6) water stress will reduce plant sensitivity to oxidant type damage; (7) plants grown under low fertility are more sensitive than those grown under high fertility levels, except plants grown on enriched nitrogen levels, which show an increasing sensitivity.

Davis and Wood (1973a), studying the influence of environmental factors on the sensitivity of Virginia pine (*Pinus virginiana* Mill.) to ozone, reported a direct relationship between relative humidity and ozone injury and a direct correlation between temperature at which plants were maintained before and after exposure and the amount of ozone injury. In contrast, an inverse relationship was observed between exposure temperature and degree of plant injury. However, Costonis and Sinclair (1969) reported that the amount of ozone injury on eastern white pine was favored by high exposure temperature.

Indicator Plants

Plants have long been recognized as sensitive indicators of air pollution and air pollutants. An excellent review paper on the subject is presented by Heck (1966). The paper discusses some general monitoring programs and the results obtained from using plants to assess the presence of phytotoxic air pollutants. The monitoring programs include: (1) field surveys by visually inspecting an area that is polluted; and (2) field monitoring using plants as sensitive monitors for a pollutant in question. Also discussed in the paper is the need for unified injury indices to evaluate air pollution injury to plants.

Many papers have been written about the use of certain plants as field monitors of particular pollutants (Benedict and Breen, 1955; Berry, 1964; Heck and Heagle, 1970; Heggestad and Darley, 1969; and Walker and Barlow, 1974). Heggestad and Darley (1969) suggest using pinto bean and/or annual bluegrass to determine the possible presence of both ozone and PAN. Spinach, radish, tomato, petunia, and white pine are also suggested to determine the presence of ozone. Berry (1964) also suggests the use of white pine to detect low levels of air pollution and to determine, by use of clones that differ in sensitivity, something about the concentration level of pollution.

A direct correlation of concentrations of ozone and tobacco weather fleck has been shown (MacDowall et al., 1964), resulting in the use of tobacco as a sensitive monitor for ozone (Heck and Heagle, 1970; Heggestad and Darley, 1969; Walker and Barlow, 1974). Heggestad and Darley (1969) used three different varieties of tobacco, one resistant,

one sensitive and one supersensitive, to indicate the presence and obtain some information levels of ozone in the field.

Several studies have suggested using native weeds to detect the presence of pollutants (Benedict and Breen, 1955; Heck, 1966; Heggestad and Darley, 1969). This will require careful screening of possible native plants until certain sensitive species or varieties are found. There may be some limitations due to the possible absence of a susceptible species or due to low soil moisture, a significant factor in reducing the sensitivity of the plant to the pollutant (Heggestad and Darley, 1969; Seidman, et al., 1965).

Certain environmental conditions have been shown to affect the use of plants as indicators of air pollution (Heggestad and Darley, 1969; Seidman et al., 1965). In general, conditions which promote optimum growth, such as high soil moisture, high humidity, balanced nutrition, and moderate or high temperatures, favor increased plant injury.

Impact of Air Pollution on Forest Ecosystems and Plant Populations

Little information is known and far less has been written about the sensitivity or response of non-crop species, plant communities, and forest ecosystems to air pollutants. A useful review is presented by Scurfield (1960) on air pollution and tree growth. Treshow (1968) presents an excellent review on the impact of air pollutants on plant populations. He states that air pollutants may modify plant community composition even though only a few species are sensitive. Major characteristics and stability of a plant community may depend on relatively few species. If these are destroyed, the

entire community may deteriorate. The air pollution threat may be particularly great in the arid regions of the west where man's survival depends on sustained water supply, which in turn is dependent upon healthy watershed cover (Treshow, 1968).

Smith (1974) concentrated on the most recent advancements in assessing the impact of air pollution on the vegetative component of forest ecosystems, and concluded that certain tree species--and the ecosystem of which they are a part--were adversely influenced by intermediate and high doses of air pollutants. The response of individual trees may range from reduced growth and reproduction through poor health and death. The ecosystem reaction may range from reduced productivity with shifts in species composition to simplification with altered ecosystem function and stability.

Miller (1973b) described the present stand composition, degree of injury, and mortality in ponderosa pine in relation to the concentrations of oxidant air pollution in a heavily damaged area on the San Bernardino National Forest in southern California. Results of his study suggest that the average ponderosa pine tree, the predominant species in the study area, suffers severe damage and is deteriorating at an alarming rate, with tree mortality ranging from 8 to 10 percent. Some possible physical and biological factors associated with oxidant-induced changes in community composition were discussed, including decreased ponderosa pine seed production and possible predisposition to injury. He concluded that the typical appearance of the forest dominated by ponderosa pine will change in ways that may reduce the recreational uses, wildlife habitat, and watershed values of the area.

Effects of Pollutants on Parasitic Diseases of Plants

At present, little is known of the effects of pollutants on parasitic diseases of plants. Heagle (1973) in reviewing the subject, states that "there is growing evidence that ozone can decrease infection, invasion, and sporulation of fungi parasitic on growing plants." Several reports are enumerated that support his theory.

It has been suggested that trees may be rendered more susceptible to infection by the root-rotting fungus, *Armillaria mellea* (Vahl.) Quel. due to air pollution exposure (Sinclair, 1969; Smith, 1974). No specific evidence is available to support this hypothesis, although it is well known that this fungus most efficiently invades trees under stress.

Evidence has been accumulated indicating that atmospheric pollution injury predisposes conifers to bark beetle infestation (Smith, 1974). Forest stands in the San Bernardino mountains of California exhibiting advanced symptoms of ozone pollution injury were most frequently infested by the western pine beetle (*Dendroctonus brevicomis* Lec.) and the mountain pine beetle (*D. ponderosae* Hopk.), (Stark et al., 1968). As severity of oxidant injury increased, live crown ratio decreased and incidence of bark beetle infestation increased.

Oxidant air pollution injury to foliage of ponderosa and Jeffrey pines increased the susceptibility of roots to infection and colonization by *Fomes annosus* (Fr.) Cke. under field and fumigation chamber experiments (James et al., 1980a). Colonization of host tissue of both species by *F. annosus* was directly related to ozone

dose and seedling injury. Increased susceptibility of stumps to *F. annosus* appeared to be associated with decreased oleoresin exudation and decreased colonization by other fungi (especially *Trichoderma* spp. and bluestain fungi) (James et al., 1980b).

Exposure Chamber Systems

The effects of air pollutants on plants have been studied by the use of a variety of exposure-chamber systems. Early exposure chambers were primarily positive pressure recirculating designs (Berry, 1970; Hill, 1967), small greenhouses (Berry, 1970; Setterstrom and Zimmerman, 1938), or a number of other facilities, including a modified walk-in type refrigerator (Menser and Heggestad, 1964). Later designs were constructed in cylinders with open tops to permit fumigations in the field with a minimum alteration of temperature, relative humidity, rainfall, and light intensity (Heagle et al., 1973; Heagle et al., 1979; Mandl et al., 1973). Recent designs incorporate the cylinder concept of others with a negative pressure, single-pass airflow gas dispensing system with an impeller for mixing introduced pollutants (Heck et al., 1978). This continuous stirred tank reactor (CSTR) design permits maintaining plants in a uniformly mixed exposure chamber regardless of inlet air velocity. Unlike earlier designs, CSTR chambers introduce pollutants to plants from the top of the chamber, similar to the way plants would be exposed under normal environmental conditions.

CHAPTER III

MATERIALS AND METHODS

Plant Material

Two- and four-year-old Rocky Mountain ponderosa pine seedlings (*Pinus ponderosa* var. *scopulorum* (Englm.)) were used in the study. Two-year-old seedlings were grown from seeds collected in the Arapahoe-Roosevelt and Pike-San Isabel National Forests of Colorado. Seeds were soaked in water for 24 hours and then planted in 4 cm diameter, 21 cm long plastic tubes containing 4:1:1 (v/v) soil : vermiculite : perlite mix. The tubes were then placed in the greenhouse where they received ambient air. Four-year-old seedlings were donated by the Colorado State Forest Service Foothills Nursery. The seed source for these seedlings was the same as the previous seedlings. Seedlings were watered as needed and fertilized with a liquid solution (3.25 ml/l) of 30N : 10P : 10K fertilizer once per week throughout the growing season (April-October).

Indicator Plants

Six species vegetatively propogated and utilized in the study were: quaking aspen (*Populus tremuloides* Michx.), plains cottonwood (*Populus sargentii* Dode.), narrowleaf cottonwood (*Populus angustifolia* James), mullein (*Verbascum thapsus* L.), gooseberry (*Ribes inerme* Rydb.), and willow (*Salix exigua* Nutt. and/or *Salix lucida* Muhl.).

Fumigations were conducted from May to October, 1981. Four to eight plants were fumigated at 0.25 and/or 0.20 ppm ozone for 24 hr/day until an estimate could be made of tissue sensitivity.

Chamber Design and Exposure Methods

Two exposure chambers were constructed using 8 mil polyvinyl chloride plastic film. The method of Heagle et al. (1973) was used, modified with a hood and vent apparatus to permit fumigations in a greenhouse. The cylindrical chambers were 1.5 m high and 1.0 m in diameter. The lower half of the chamber was covered by a double layer of film, with the inner layer perforated with 108 holes, 1 cm in diameter. The system for introducing air consisted of a blower in a wooden box containing a 4-inch thick fiberglass particulate filter and a 1-inch thick charcoal filter. Ozone or charcoal filtered air was introduced into the chambers via the lower bilayer of plastic. Ozone was generated from reconstituted air (nitrogen and oxygen) by electric discharge with an ozone generator (Model 03V5-0, Ozone Research and Equipment Corp., Phoenix, AZ 86019). Ozone was monitored continuously with a Dasibi ozone monitor (Model 1003 AH, Dasibi Environmental Corp., Glendale, CA 91205). Temperature (Model 701, Yellow Springs Instrument Co., Yellow Springs, OH 45387) and relative humidity (Model RH-515-A with HS-3552-B humidity sensor, Hy-Cal Engineering, Santa Fe Springs, CA 90670) were monitored continuously. All parameters were continuously recorded on a strip chart recorder (Model MS 413B, Easterline Angus Instrument Corp., Indianapolis, IN 46224).

Experiment 1

This experiment was designed to provide a rough estimate of the symptomatic response of Rocky Mountain ponderosa pine to various ozone concentrations and fumigation durations. Exposures were conducted at 1.0, 0.75, 0.50, and 0.25 ppm[1] ozone for 24 hr/day until an estimate could be made of tissue sensitivity. Five seedlings were used in the fumigation and control chambers for each ozone concentration. Fumigations were conducted from April until July, 1981. Seedlings were watered and examined for injury symptoms daily. Temperature and relative humidity were not held constant, but were similar between control and fumigation chambers. The mean temperature of the chambers was 24.6 (±6.1)[2] C and the mean RH was 62.4 (± 12.3) percent.

Experiment 2

The second experiment was designed to determine the threshold ozone concentration for visual injury to Rocky Mountain ponderosa pine. Exposures were conducted at 0.50, 0.40, 0.30, or 0.25 ppm ozone during daylight hours from 0800 to 1800 for 21 consecutive days from July to October, 1981, and in May 1982. Forty seedlings were used for each exposure concentration, 20 seedlings in the ozone chamber and 20 seedlings acting as controls in an identical chamber minus the ozone. Half of the seedlings in each chamber were grown from seed collected in the Pike-San Isabel National Forests, the other half from the Arapahoe-Roosevelt National Forests. Seedlings were watered and

---

[1]All ozone fumigation concentrations are ± 0.05 ppm.

[2]Numbers in parentheses are standard deviations.

examined for visual ozone injury symptoms each day prior to ozone introduction. Each seedling was given a subjective visual rating of the two prominent symptoms displayed, mottling and chlorosis. Four severity classes were utilized for mottling: no response, slight, moderate, and severe. Three classes were recognized for chlorosis: no response, mild, and severe. Temperature and relative humidity were not held constant, but were similar between control and fumigation chambers. The mean temperature of the chambers was 23.5 (± 4.5)[3] C and the mean RH was 64.0 (± 8.0) percent.

Experiment 3

Loss of chlorophyll has been used as a measure of the impairment of leaf function of beans (Todd and Arnold, 1961) and pines (Miller et al, 1963) injured by air pollutants. For this reason, chlorophyll extractions were made from each year's needles of four-year-old Rocky Mountain ponderosa pine seedlings fumigated at 0.50 ppm ozone during daylight hours for 9 hr/day (0900 to 1800) for 18 days. Extractions were also made from unfumigated control seedlings. Two repetitions were performed for each needle age. Extractions were performed 3 and 9 days after the seedlings were fumigated. Extraction techniques were those described by Comar (1942) and Comar et al. (1943), except that 2.50 g of fresh leaf tissue were ground in a mortar with 85 percent acetone to which a small amount of calcium carbonate had been added. The macerate was vacuum filtered through Whatman No. 1 grade filter paper. The residue was

[3]Numbers in parentheses are standard deviations.

ground again in 85 percent acetone until a white mash remained and refiltered. All filtrates were combined and made to a known volume with 85 percent acetone and the optical density immediately measured at 655 mu with a Bausch and Lomb Spectronic 20 spectrophotometer. Chlorophyll concentration determinations and the percentage of controls were calculated for each year's needles. Chlorophyll concentrations expressed as a percentage of controls were compared between needle age and time of extraction using a two-way analysis of variance. Multiple comparisons were performed utilizing confidence intervals ($P = 0.05$).

Ozone Field Monitoring

Ozone monitoring was conducted for 2-wk periods in the ponderosa pine region of the Colorado Front Range using a Dasibi ultraviolet absorption photometer. Two sites were chosen for continuous oxidant recordings. They were (a) Conifer, 32 km (20 mi) southwest of Denver, elevation approximately 2918 m (8000 ft) from 25 October to 8 November 1981, and (b) Rist Canyon, 24 km (15 mi) west of Fort Collins, elevation approximately 2371 m (6500 ft) from 9 September to 22 September 1981. Monitoring was performed 1 to 2 m from the ground.

CHAPTER IV

RESULTS

Experiment 1

Fumigations at 1.0 and 0.75 ppm ozone for 48 hours produced acute symptoms of needle banding, tip necrosis, premature senescence and needle abscission. Additionally, chronic symptoms occurred including chlorotic mottling and entire needle chlorosis. Only the chronic responses of mottling and chlorosis were noted after 8 days fumigation at 0.50 ppm (Figure 1). No seedlings were symptomatic at 0.25 ppm ozone after 21 days of continuous fumigation.

Experiment 2

The threshold ozone concentration for visual injury to ponderosa pine seedlings apparently lies between 0.25 and 0.30 ppm for a 10 hr/day, 21 day exposure (Table 1). Prolonged exposure to 0.25 ppm ozone failed to produce visible symptoms on any of the 20 fumigated seedlings. Concentrations of ozone at 0.30 to 0.50 ppm produced a distinct type of chronic chlorotic mottling and, in addition, a general yellowing or chlorosis eventually leading to premature senescence and needle abscission (Figure 1). Mottling and entire needle chlorosis were considerably less pronounced on the 0.30 ppm seedlings when compared to the seedlings exposed at 0.50 and 0.40 ppm (Table 1). The 0.50 and 0.40 ppm fumigations produced similar symptom severity for both mottling

Figure 1. Needles of ponderosa pine fumigated at 0.50 ppm for 21 days, 10 hr/day (0800-1800); (left to right) control, chlorotic mottling, slight chlorosis, severe chlorosis.

Table 1. Percentage of seedlings displaying different degrees of mottling and chlorosis when fumigated at four ozone concentrations.[a]

Ozone Concentration (ppm)[c]	Percentage of Seedlings Displaying Symptoms[b]						
	Mottling				Chlorosis		
	None	Slight	Moderate	Severe	None	Mild	Severe
0.50	5	70	25	0	20	65	15
0.40	0	70	25	5	30	60	10
0.30	50	45	5	0	70	25	5
0.25	100	0	0	0	100	0	0

[a]Fumigations conducted 10 hr/day for 21 days (0800 to 1800). Symptoms reported as of the 21st day.

[b]n = 20 for the one replication performed at each ozone concentration.

[c]All ozone concentrations are ± 0.05 ppm.

and chlorosis (Table 1). No apparent difference in response was noted for the two different seed sources utilized, therefore the results were combined. No statistical analysis was performed on this data. No mottling or chlorotic symptoms were observed on any control chamber seedlings.

Response time, or time from start of fumigation until symptom expression, also implicated 0.25 to 0.30 ppm ozone as the threshold concentration for visual injury to ponderosa pine (Figs. 2 and 3). Mottling (Fig. 2) was the most prominent response noted because symptom expression occurred sooner than for chlorosis (Fig. 3) and a greater percentage of seedlings were symptomatic at the end of the 21 day fumigation for each ozone concentration. Mottling response times were similar for the 0.50 and 0.40 ppm fumigations, but a definite increase in response time can be seen for the 0.30 ppm fumigation (Fig. 2).

A more gradual decreasing trend can be seen for the chlorosis response time with regard to ozone fumigation concentration (Fig. 3). After a 21 day fumigation at 0.50, 0.40, or 0.30 ppm ozone the percentage of seedlings displaying chlorotic needles were 80, 70, and 30 respectively. No seedlings were symptomatic at 0.25 ppm ozone.

Experiment 3

Chlorophyll extractions on the needles of 0.50 ppm fumigated seedlings (9 hr/day (0900 to 1800) for 18 days) provided a good means of quantifying and confirming the visual observations of chlorotic mottling and needle chlorosis (Table 2). The extractions performed 3 days after seedling removal from the fumigation chamber showed a reduction in chlorophyll for all four needle ages. The results

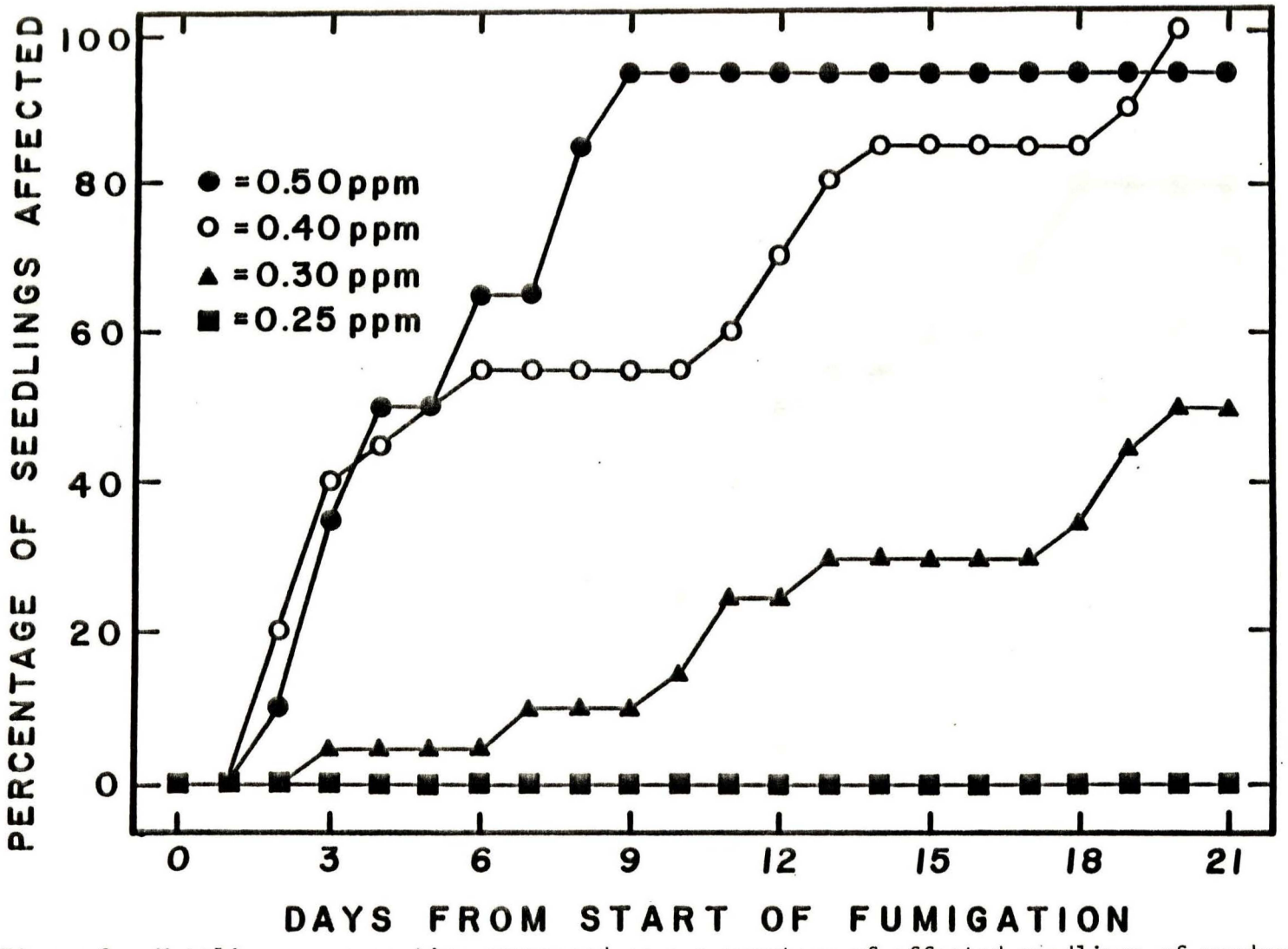

Figure 2. Mottling response time expressed as a percentage of affected seedlings of ponderosa pine fumigated at four different ozone concentrations. Fumigations conducted for 10 hr/day (0800-1800) for 21 days.

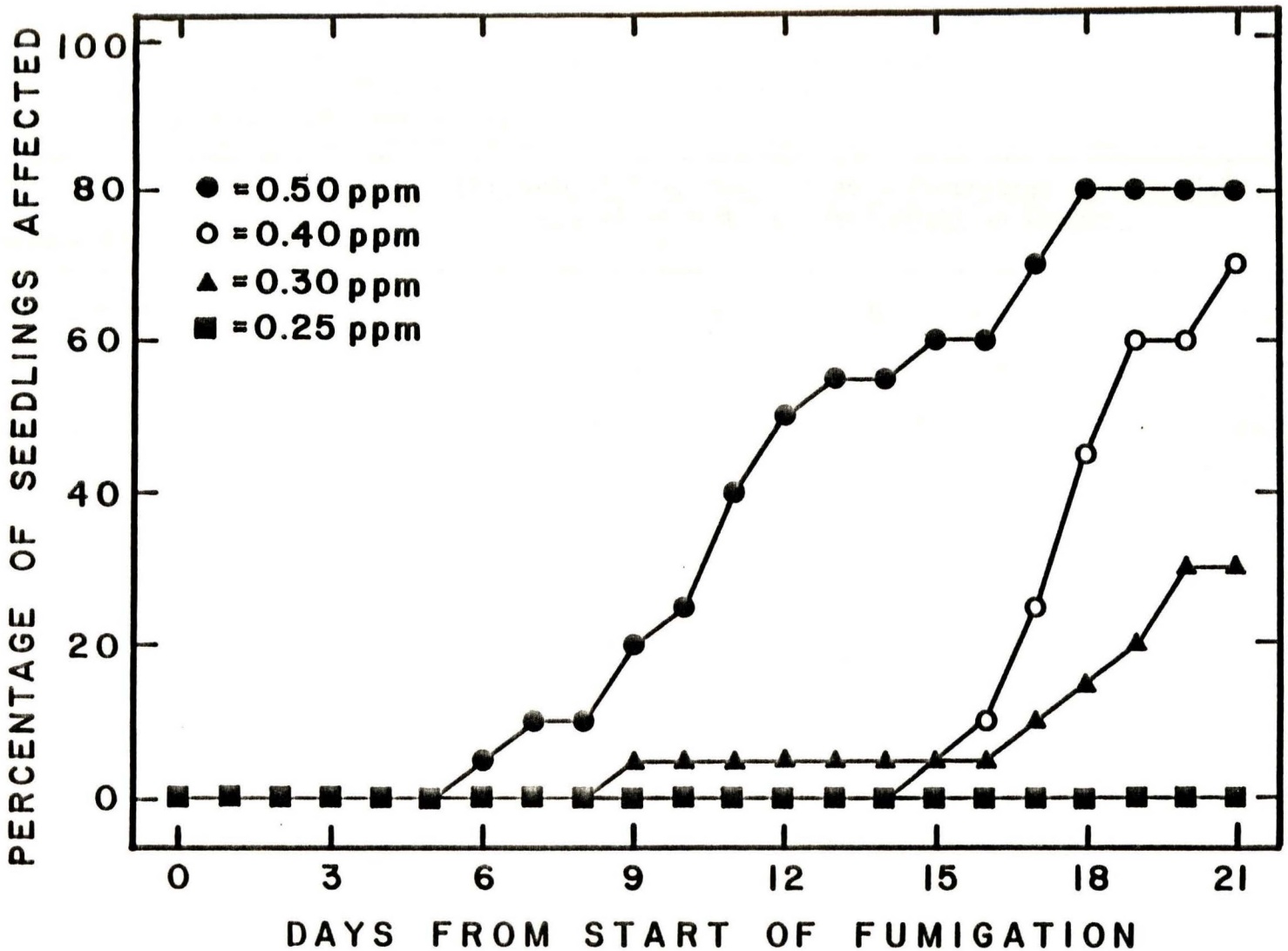

Figure 3. Chlorosis response time expressed as a percentage of affected seedlings of ponderosa pine fumigated at four different ozone concentrations. Fumigations conducted for 10 hr/day (0800-1800) for 21 days.

Table 2. Chlorophyll concentrations, expressed as a percentage of controls, from needles of four-year-old Rocky Mountain ponderosa pine seedlings fumigated at 0.50 ppm ozone for 9 hr/day (0900-1800) for 18 days.

Needle Age	Chlorophyll Concentration as a Percentage of Controls[a] Days after Removal from Fumigation Chamber 3	9	Means
1982 current	82.5 ± 9.6	69.1 ± 9.6	75.8 ± 6.8
1981 1 year	77.9 ± 9.6	67.4 ± 9.6	72.6 ± 6.8
1980 2 year	94.2 ± 9.6	73.7 ± 9.6	84.0 ± 6.8
1979 3 year	85.8 ± 9.6	65.1 ± 9.6	75.4 ± 6.8
Means	85.1 ± 4.8[b]	68.8 ± 4.8	

[a]Data are the mean and confidence intervals (P = 0.05) of two replicates; chlorophyll concentrations expressed as a percentage of that year's unfumigated controls.

[b]Column and row means may not calculate out exactly due to rounding.

were significant, when compared to controls, for all needle ages except the two-year-old needles. When the extractions were performed 9 days after fumigation, reductions in chlorophyll were significant for all four needle ages. Chlorophyll concentrations 9 days after fumigation were significantly less than concentrations 3 days after fumigation for all four needle ages. No significant difference in chlorophyll reduction was found when comparing needle ages within the 3 days or 9 days after fumigation extractions.

Indicator Plants

Of the 6 plant species studied, willow and aspen appear to be the most promising for use as indicator plants. At 0.20 ppm ozone for 8 hours willow had tan to brown bifacial necrotic lesions along the margins on the oldest leaves, with symptom severity decreasing acropetally from older to younger leaves. After 24 hours of continuous fumigation the necrotic areas on the leaves were much larger, with the affected areas covering approximately 50 percent of the oldest leaf surfaces. Premature senescence, leaf curling and abscission of older leaves had begun after 48 hours of ozone fumigation. Symptom severity decreased acropetally, with the youngest, immature leaves near the growing tip resistant to ozone injury.

At 0.20 ppm, aspen leaves began to show black, bifacial necrotic spots after 10 hours of ozone fumigation. Older leaves were susceptible, younger leaves appeared resistant. Lesions were more extensive, covering approximately 20 percent of the leaf surface area, after 24 hours of continuous fumigation. Lesions frequently extended across the veins. Symptom severity was only slightly more advanced

after 48 hours of fumigation. No discernible changes were apparent after 72 hours of continuous ozone fumigation.

Narrowleaf cottonwood was moderately susceptible, showing small black interveinal necrotic spots on older leaves after 24 hours of continuous fumigation at 0.20 ppm. Younger leaves were resistant. No change was discernible after 48 or 72 hours of fumigation.

Plains cottonwood, mullein, and gooseberry were not susceptible to ozone at 0.25 ppm, displaying no symptoms after 1 week of continuous fumigation.

CHAPTER V

DISCUSSION

Rocky Mountain ponderosa pine seedlings are sensitive to controlled ozone fumigations. Concentrations of ozone at 0.30 to 0.50 ppm produced a chlorotic mottling visibly similar to that found on ponderosa pine growing under natural conditions in the forests of southern California (Miller et al., 1963; Richards et al., 1968). Results of chlorophyll extractions performed on fumigated seedlings confirmed the visual observations of mottling and chlorosis.

Needle age has been reported to be an important factor in determining sensitivity of pine needles to ozone. Conflicting results have been reported. Davis and Wood (1972), in studying the relative susceptibility of 18 coniferous species to ozone, stated that current foliage was more sensitive than older foliage. In contrast, Miller (1973a) reported that coastal ponderosa pine one-year-old needles were injured three times as severely as were current year needles. Neither of these findings were observed with this study. No significant difference in chlorophyll reduction was found when comparing needle ages on ozone fumigated Rocky Mountain ponderosa pine.

The threshold for visual injury to ponderosa pine apparently lies between 0.25 and 0.30 ppm for a 10 hr/day fumigation over 21 days. Fumigations at 0.50 and 0.40 ppm were similar with regard to the percent of seedlings affected and severity of response.

Seedlings fumigated at 0.30 ppm had a less severe response and fewer seedlings were affected. No response was elicited at 0.25 ppm ozone. This does not imply that ozone concentrations less than 0.30 ppm would not induce injury, or that seedlings not visibly symptomatic after a 21 day fumigation are resistant to ozone injury. Miller et al. (1969) has shown that decreases in apparent photosynthesis rate during 0.15 and 0.30 ppm ozone fumigations to coastal ponderosa pine detected functional impairment before chlorotic mottle symptoms were visible. Evans and Miller (1972a) found histological and histochemical changes in coastal ponderosa pine occurring within 5 to 7 days, but visual symptoms were not evident until 2 to 3 weeks after ozone fumigation at 0.45 ppm.

It has been suggested that the Rocky Mountain variety of ponderosa pine may be more resistant to oxidant injury than the coastal variety (James and Staley, 1980). It is not possible, using the results of this study, to ascertain whether this is the case. Many variables, including plant age, environmental conditions and differing experimental designs makes comparisons to previous field and laboratory studies difficult. It is interesting, however, that results similar to mine were observed on coastal ponderosa pine, specifically that typical chlorotic mottle needle symptoms appeared after 3 to 4 weeks fumigation with 0.30 ppm ozone for 9 hr/day (Miller and Parmeter, 1965). Rocky Mountain ponderosa pine may be less sensitive to oxidant injury in the field because it has been shown that plants grown under semi-drought conditions, such as that experienced along the Colorado Front Range, are less sensitive than those grown with adequate water (Heck et al., 1965).

Oxidant levels within Denver are sufficient to cause plant injury (Anderson et al., 1977; Colorado Department of Health, 1980b; James and Staley, 1980). Knowledge of oxidant levels in nearby forested areas is fragmentary due to insufficient monitoring. Limited ozone monitoring was done by the Colorado Department of Health during 1979-1980 at selected locations in the mountains of Colorado. Results of two of the highest ozone sites found, Conifer (32 km (20 mi) southwest of Denver) and Silver Plume (64 km (40 mi) west of Denver), indicated 0.03 and 0.04 ppm for average hourly concentrations and 0.06 and 0.05 ppm for average daily peak ozone concentrations, respectively. The highest ozone level recorded was 0.09 ppm in Conifer. Preliminary monitoring done during this study for 2-wk intervals at two locations along the forested Front Range of Colorado indicated average hourly concentrations of 0.05 ppm with corresponding average daily peak oxidant concentrations of 0.07 ppm. The highest concentration recorded was again found in Conifer, specifically 0.09 ppm. Monitoring was conducted during September through November. Denver's highest ozone levels, however, generally occur during summer when high temperatures and strong sunlight promote photochemical reactions (Anderson et al., 1977). An expanded monitoring program encompassing several known pollution locations conducted from April through October would be necessary to ascertain whether oxidant pollution levels in Colorado's forested areas are sufficient to cause injury to ponderosa pine.

Indicator plants have been proven useful as inexpensive monitors of phytotoxic air pollutants. Preliminary results of this study indicate that willow and aspen may be useful as native plant indicators of high levels of oxidant air pollution along the Colorado Front Range.

Field surveys conducted during 1978 and 1979 within and adjacent to the Denver Metropolitan area found very few trees displaying typical oxidant air pollution symptoms (James and Staley, 1980). Most injured trees were southeast of Denver. Symptoms observed included shortened needles, fewer needles, and chlorotic banding. No chlorotic mottling typical of seedlings fumigated in this study or oxidant-damaged trees in California was found. Five permanent evaluation plots were established including some trees displaying slight air pollution injury.

The results of my study indicate that ponderosa pine seedlings are sensitive to oxidant air pollution in controlled greenhouse fumigations. The potential for air pollution damage to ponderosa pine around Denver cannot be determined with these results. Whether oxidant pollutants generated in the Denver Metropolitan area are reaching forested areas is not known but is strongly suspected. Although upslope-downslope diurnal wind patterns typical of mountain slopes may cause pollutants to reach the forested areas west of Denver (James and Staley, 1980), prevailing westerly winds tend to carry most pollution east, away from ponderosa pine stands. The potential for oxidant damage to ozone sensitive vegetation therefore appears to be greatest within and to the east of the metropolitan areas along the Front Range. Only after an expanded monitoring program is employed coupled with field fumigations on ponderosa pine will it be possible to make valid conclusions regarding the potential for air pollution damage to Rocky Mountain ponderosa pine.

LITERATURE CITED

Adams, D.F., Mayhew, D.J., Gnagy, R.M., Richey, E.P., Koppe, R.K., and Allen, I.W. 1952. Atmospheric pollution in the ponderosa pine blight area. Ind. Eng. Chem. 44:1356-1365.

Adams, D.F., Shaw, C.G., and Yerkes, W.D., Jr. 1956. Relationship of injury indexes and fumigation fluoride levels. Phytopathology 46:587-591.

Anderson, G.E., Hayes, S.R., Hillyer, M.J., Killus, J.P. and Mundkur, P.V. 1977. Air quality in the Denver Metropolitan Region, 1974 - 2000. Systems Applications, Inc., U.S. Environ. Pro. Agency Report 908/1-77-002. 406 p.

Asher, J.E. 1956. Observations and theory on X-disease or needle dieback. Unpublished report to Arrowhead Ranger District, San Bernardino National Forest.

Barnes, R.L. 1972a. Effects of chronic exposure to ozone on photosynthesis and respiration of pines. Environ. Pollut. 3:133-138.

Barnes, R.L. 1972b. Effects of chronic exposure to ozone on soluble sugar and ascorbic acid contents of pine seedlings. Can. J. Bot. 50:215-219.

Benedict, H.M., and Breen, W.H. 1955. The use of weeds as a means of evaluating vegetation damage caused by air pollution. In Proc. Third Nat'l. Air Poll. Conf., Pasadena, Calif. pp. 177-190.

Berry, C.R. 1961. White pine emergence tipburn, a physiogenic disturbance. Southeastern Forest Exp. Sta. Paper 130. 8 p.

Berry, C.R. 1964. Eastern white pine, a tool to detect air pollution. Southern Lumberman 209:164-166.

Berry, C.R. 1970. A plant fumigation chamber suitable for forestry studies. Phytopathology 60:1613-1615.

Berry, C.R. 1974. Age of pine seedlings with primary needles affects sensitivity to ozone and sulfur dioxide. Phytopathology 64:207-209.

Berry, C.R., and Ripperton, L.A. 1963. Ozone, a possible cause of white pine emergence tipburn. Phytopathology 53:552-557.

Colorado Department of Health. 1977. Analysis of air quality in the Denver air quality maintenance area. Air Pollution Control Division, Denver, CO. 148 p.

Colorado Department of Health. 1980a. Colorado air quality data report--1980. Air Pollution Control Division, Denver, CO. 58 p.

Colorado Department of Health. 1980b. Report to the Public--1980. Vol. 1: Activity Summary. Air Quality Control Commission, Denver, CO 119 p.

Comar, C.L. 1942. Analysis of plant extracts for chlorophylls a and b. Ind. Eng. Chem., Anal. Ed. 14:877-879.

Comar, C.L., Benne, E.J., and Buteyn, E.K. 1943. Calibration of a phytoelectric colorimeter for the determination of chlorophyll. Ind. Eng. Chem., Anal. Ed. 15:524-526.

Costonis, A.C., and Sinclair, W.A. 1969. Relationship of atmospheric ozone to needle blight of eastern white pine. Phytopathology 59:1566-1574.

Coyne, P.I., and Bingham, G.E. 1981. Comparative ozone dose response of gas exchange in a ponderosa pine stand exposed to long-term fumigations. J. Air Poll. Contr. Assoc. 31:38-41.

Darley, E.F. 1968. Introductory remarks for the symposium on trends in air pollution damage to plants. Phytopathology 58:1075.

Davis, D.D. 1975. Resistance of young ponderosa pine seedlings to acute doses of PAN. Plant Dis. Reptr. 59:183-184.

Davis, D.D. 1977. Response of ponderosa pine primary needles to separate and simultaneous ozone and PAN exposures. Plant Dis. Reptr. 61:640-644.

Davis, D.D., and Coppolino, J.B. 1974. Relationship between age and ozone sensitivity of current needles of ponderosa pine. Plant Dis. Reptr. 58:660-663.

Davis, D.D., and Wood, F.A. 1972. The relative susceptibility of eighteen coniferous species to ozone. Phytopathology 62:14-19.

Davis, D.D., and Wood, F.A. 1973a. The influence of environmental factors in the sensitivity of Virginia pine to ozone. Phytopathology 63:371-376.

Davis, D.D., and Wood, F.A. 1973b. The influence of plant age on the sensitivity of Virginia pine to ozone. Phytopathology 63:381-388.

Evans, L.S., and Miller, P.R. 1972a. Ozone damage to ponderosa pine: a histological and histochemical appraisal. Amer. J. Bot. 59: 297-304.

Evans, L.S., and Miller, P.R. 1972b. Comparative needle anatomy and relative ozone sensitivity of four pine species. Can. J. Bot. 50:1067-1071.

Evans, L.S. and Miller, P.R. 1975. Histological comparison of single and additive O_3 and SO_2 injuries to elongating ponderosa pine needles. Amer. J. Bot. 62:416-421.

Freebairn, H.T. 1960. The prevention of air pollution damage to plants by use of vitamin C sprays. J. Air Poll. Contr. Assoc. 10: 314-317.

Heagle, A.S. 1973. Interactions between air pollutants and plant parasites. Ann. Rev. Phytopathol. 11:365-388.

Heagle, A.S., Body, D.E., and Heck, W.W. 1973. An open-top field chamber to assess the impact of air pollution on plants. J. Environ. Quality 2:365-368.

Heagle, A.S., Philbeck, R.B., Rogers, H.H., and Letchworth, M.B. 1979. Dispensing and monitoring ozone in open-top field chambers for plant-effects studies. Phytopathology 69:15-20.

Heck, W.W. 1966. The use of plants as indicators of air pollution. Int. J. Air Water Pollut. 10:99-111.

Heck, W.W., Dunning, J.A., and Hindawi, I.J. 1965. Interactions of environmental factors on the sensitivity of plants to air pollution. J. Air Poll. Contr. Assoc. 15:511-515.

Heck, W.W., and Heagle, A.S. 1970. Measurement of photochemical air pollution with a sensitive monitoring plant. J. Air Poll. Contr. Assoc. 20:97-99.

Heck, W.W., Philbeck, R.B., and Dunning, J.A. 1978. A continuous stirred tank reactor (CSTR) system for exposing plants to gaseous air contaminants: Principles, specifications, construction, and operation. Agric. Res. Serv., Series No. 181. 32 p.

Heggestad, H.E. 1968. Diseases of crops and ornamental plants incited by air pollutants. Phytopathology 58:1089-1097.

Heggestad, H.E., and Darley, E.F. 1969. Plants as indicators of the air pollutants ozone and PAN. In Eur. Congr. Influence Air Pollution Plants Anim. Proc. 1st:329-335.

Hepting, G.H. 1964. Damage to forests from air pollution. J. of Forestry 62:630-634.

Hepting, G.H. 1966. Air pollution impact on some important species of pine. J. Air Poll. Contr. Assoc. 16:63-65.

Hill, A.C. 1967. A special purpose plant environmental chamber for air pollution studies. J. Air Poll. Contr. Assoc. 17:743-748.

Hill, A.C., Pack, M.R., Treshow, M., Downs, R.J., and Transtrum, L.G. 1961. Plant injury induced by ozone. Phytopathology 51:356-363.

James, R.L., Cobb, F.W., Jr., Miller, P.R., and Parmeter, J.R., Jr. 1980a. Effects of oxidant air pollution on susceptibility of pine roots to *Fomes annosus*. Phytopathology 70:560-563.

James, R.L., Cobb, F.W., Jr., Wilcox, W.W., and Rowney, D.L. 1980b. Effects of photochemical oxidant injury of ponderosa and Jeffrey pines on susceptibility of sapwood and freshly cut stumps to *Fomes annosus*. Phytopathology 70:704-708.

James, R.L., and Staley, J.M. 1980. Photochemical air pollution damage survey of ponderosa pine within and adjacent to Denver, Colorado: a preliminary report. USDA, For. Serv., For. Insect and Disease Mgmt., Bio. Evaluation R2-80-6. 21 p.

Karpen, D.N. 1970. Ozone and sulfur dioxide synergism: foliar injury to a ponderosa pine geographic race plantation in the Puget Sound region. Plant Dis. Reptr. 54:945-948.

Linzon, S.N. 1960. The development of foliar symptoms and the possible cause and origin of white pine needle blight. Can. J. Bot. 38:153-161.

Linzon, S.N. 1966. Damage to eastern white pine by sulfur dioxide, semimature tissue needle blight, and ozone. J. Air Poll. Contr. Assoc. 16:140-144.

Linzon, S.N. 1967. Ozone damage and semimature-tissue needle blight of eastern white pine. Can. J. Bot. 45:2047-2061.

MacDowall, F.D.H., Mukammal, E.I., and Cole, A.F.W. 1964. Direct correlation of air pollution ozone and tobacco weather fleck. Can. J. Pl. Sci. 44:410.

Mandl, R.H., Weinstein, L.H., McCune, D.C., and Keveny, M. 1973. A cylindrical open top chamber for exposure of plants to air pollutants in the field. J. Environ. Qual. 2:371-376.

McCormick, R.A. 1968. Air pollution climatology. In Stearn, A.C. (ed.). Air Pollution. Vol. I. (2nd ed.). Academic Press, New York and London. p. 275-320.

Menser, H.A., and Heggestad, H.E. 1964. A facility for ozone fumigation of plant materials. Crop. Sci. 4:103-105.

Miller, P.R. 1973a. Susceptibility to ozone of selected western confiers. 2nd Int. Cong. Plant Pathol. Abstr. No. 0579.

Miller, P.R. 1973b. Oxidant-induced community change in a mixed conifer forest. Adv. Chem. Ser. No. 122. pp. 101-117.

Miller, P.R., and Evans, L.S. 1974. Histopathology of oxidant injury and winter fleck injury on needles of western pines. Phytopathology 64:801-806.

Miller, P.R., and Parmeter, J.R., Jr. 1965. Effect of sustained low-concentration ozone fumigation on ponderosa pine (Abst.) Phytopathology 55:1068.

Miller, P.R., Parmeter, J.R., Jr., Flick, B.H., and Martinez, C.W. 1969. Ozone dosage response of ponderosa pine seedlings. J. Air Poll. Contr. Assoc. 19:435-438.

Miller, P.R., Parmeter, J.R., Jr., Taylor, O.C., and Cardiff, E.A. 1963. Ozone injury to the foliage of *Pinus ponderosa*. Phytopathology 53:1072-1076.

Mudd, J.B., and Kozlowski, T.T. 1975. Responses of Plants to Air Pollution. Academic Press, New York. 383 p.

Naegele, J.A. 1973. Air pollution damage to vegetation. Adv. Chem. Ser. No. 122, 137 p.

Parmeter, J.R., Jr., Bega, R.V., and Neff, T. 1962. A chlorotic decline of ponderosa pine in southern California. Plant Dis. Reptr. 46:269-273.

Rich, S. 1964. Ozone damage to plants. Ann. Rev. Phytopath. 2: 253-266.

Richards, B.L., and Taylor, O.C. 1965. Significance of atmospheric ozone as a phytotoxicant. J. Air Poll. Contr. Assoc. 15:191-193.

Richards, B.L., Sr., Taylor, O.C., and Edmunds, G.F., Jr. 1968. Ozone needle mottle of pine in southern California. J. Air Poll. Contr. Assoc. 18:73-77.

Riehl, H. and Crow, L.W. 1962. A study of Denver air pollution. Dept. Atmos. Sci. Tech. Report 33. Colorado State University, Ft. Collins, CO 15 p.

Riehl, H. and Herkhof, D. 1970. Weather factors in Denver air pollution. Dept. Atmos. Sci. Paper No. 158. Colorado State University, Ft. Collins, CO 18 p.

Scheffer, T.C., and Hedgcock, G.G. 1955. Injury to northwestern forest trees by sulfur dioxide from smelters. U.S. Dept. of Agr. Tech. Bull. 1117. 49 p.

Scurfield, G. 1960. Air pollution and tree growth. For. Abst. 21: 339-347, 517-528.

Setterstrom, C., and Zimmerman, P.W. 1938. Apparatus for studying effects of low concentrations of gases on plants and animals. Contrib. Boyce Thompson Inst. 9:161-169.

Shaw, C.G., Fisher, G.W., Adams, D.F., and Adams, M.F. 1951. Fluorine injury to ponderosa pine. Phytopathology 41:943.

Siedman, G., Hindawi, I.J., and Heck, W.W. 1965. Environmental conditions affecting the use of plants as indicators of air pollution. J. Air Poll. Contr. Assoc. 15:168-171.

Sinclair, W.A. 1969. Polluted air: Potent new selective force in forests. J. Forestry 67:305-309.

Smith, W.H. 1974. Air pollution--effects on the structure and function of the temperate forest ecosystem. Environ. Pollut. 6: 111-129.

Solberg, R.A., Adams, D.F., and Ferchau, H.A. 1955. Some effects of hydrogen fluoride on the internal structure of *Pinus ponderosa* needles. Proc. 3rd Nat'l. Air Pollut. Symp. 164-176.

Stark, R.W., Miller, P.R., Cobb, F.W., Jr., Wood, D.L., and Parmeter, J.R., Jr. 1968. Photochemical oxidant injury and bark beetle (Coleoptera:Scolytidae) infestation of ponderosa pine, I. Incidence of bark beetle infestation in injured trees. Hilgardia 39:121-126.

Taylor, O.C., and Richards, B.L., Jr. Annual Reports 1959 and 1960. Agricultural Experiment Project #1663, Citrus Research Center and Agricultural Experiment Sta., Riverside, California.

Tingey, D.T., Wilhour, R.G., and Standley, C. 1976. The effect of chronic ozone exposures on the metabolite content of ponderosa pine seedlings. Forest Sci. 22:234-241.

Todd, G.W., and Arnold, W.N. 1961. An evaluation of the methods used to determine injury to plant leaves by air pollutants. Bot. Gaz. 123:151-154.

Treshow, M. 1968. The impact of air pollutants on plant populations. Phytopathology 58:1108-1113.

Treshow, M. 1970. Ozone damage to plants. Environ. Pollut. 1:155-161.

Walker, J.T., and Barlow, J.C. 1974. Response of indicator plants to ozone levels in Georgia. Phytopathology 64:1122-1127.

Walter, H.J. 1956. Variation in sensitivity to atmospheric fluorides among ponderosa pine seedlings. Phytopathology 46:637.

Wilkinson, T.G., and Barnes, R.L. 1973. Effects of ozone on $^{14}CO_2$ fixation patterns in pine. Can. J. Bot. 51:1573-1578.

Williams, W.T., Brady, M., and Willison, S.C. 1977. Air pollution damage to the forests of the Sierra Nevada mountains of California. J. Air Poll. Contr. Assoc. 27:230-234.

APPENDICES

Appendix 1. Mean daily temperatures (C) monitored within the fumigation chamber.*

Days from Start of Fumigation	Ozone Concentration (Dates of Fumigation)							
	1.0 ppm (4/28-4/30/81)	0.75 ppm (4/30-5/2/81)	0.50 ppm (6/9-6/17/81)	0.25 ppm (6/17-7/8/81)	0.50 ppm (7/17-8/7/81)	0.40 ppm (8/11-9/1/81)	0.30 ppm (9/26-10/16/81)	0.25 ppm (5/6-5/27/82)
1	22.5	21.7	26.8	23.0	25.4	22.8	19.0	21.3
2	22.6	24.8	25.5	21.9	25.4	23.3	21.2	22.8
3	24.3	29.3	27.5	26.3	24.9	23.4	23.0	22.3
4			27.3	27.0	27.8	24.0	24.4	22.8
5			21.0	25.3	28.7	21.8	23.9	22.9
6			17.7	26.8	27.0	22.9	22.3	20.1
7			19.7	23.5	26.0	23.7	25.0	19.3
8			23.4	26.3	24.1	25.5	24.2	21.6
9				25.1	23.0	25.6	23.5	22.6
10				24.2	20.2	22.0	24.1	22.5
11				25.2	23.8	26.1	25.1	21.5
12				23.4	25.5	22.6	25.1	24.8
13				23.4	25.3	24.6	24.6	23.7
14				28.2	23.9	21.5	23.5	23.0
15				27.3	25.1	21.6	25.3	21.7
16				22.6	23.2	24.4	25.3	22.5
17				22.5	26.1	23.5	25.0	21.3
18				24.8	27.3	24.0	22.5	20.5
19				26.7	25.9	22.7	23.3	16.8
20				27.6	27.4	23.2	22.0	16.2
21				26.6	24.1	18.9	22.3	19.9

*Daily means are calculated from 8:00 A.M. to 7:00 A.M. the following day.

Appendix 2. Mean daily relative humidity monitored within the fumigation chamber.*

Days from Start of Fumigation	Ozone Concentration (Dates of Fumigation)							
	1.0 ppm (4/28-4/30/81)	0.75 ppm (4/30-5/2/81)	0.50 ppm (6/9-6/17/81)	0.25 ppm (6/17-7/8/81)	0.50 ppm (7/17-8/7/81)	0.40 ppm (8/11-9/1/81)	0.30 ppm (9/26-10/16/81)	0.25 ppm (5/6-5/27/82)
1	51.6	53.8	54.2	56.9	66.8	70.9	65.2	50.1
2	51.1	48.5	61.7	63.3	66.8	76.5	62.9	53.4
3	45.0	42.9	60.2	55.3	61.8	70.5	60.6	54.5
4			54.5	55.0	59.9	69.8	57.2	50.0
5			60.0	63.4	58.6	72.1	56.3	55.1
6			62.5	58.0	68.2	72.0	57.3	62.7
7			56.6	65.6	62.4	68.9	54.6	66.6
8			50.6	62.7	66.8	66.8	58.2	61.9
9				69.0	72.1	65.1	61.0	57.9
10				74.2	77.9	71.0	58.1	60.2
11				67.7	67.6	68.1	57.7	61.6
12				71.7	62.4	72.2	56.4	58.4
13				77.7	61.2	67.2	55.5	55.7
14				68.5	61.4	73.5	58.0	56.2
15				76.7	66.3	73.7	54.5	59.0
16				77.4	70.5	72.6	56.8	57.7
17				76.3	65.8	71.3	54.8	65.8
18				67.5	62.4	69.9	64.5	69.8
19				62.9	69.1	70.8	61.7	69.8
20				63.2	69.3	64.8	67.8	67.9
21				61.8	69.7	77.2	65.5	66.4

*Daily means are calculated from 8:00 A.M. to 7:00 A.M. the following day.

Appendix 3. Mean hourly temperature (C) monitored within the fumigation chamber.

Hour of Day*	Ozone Concentration (Dates of Fumigation)							
	1.0 ppm (4/28-4/30/81)	0.75 ppm (4/30-5/2/81)	0.50 ppm (6/9-6/17/81)	0.25 ppm (6/17-7/8/81)	0.50 ppm (7/17-8/7/81)	0.40 ppm (8/11-9/1/81)	0.30 ppm (9/26-10/16/81)	0.25 ppm (5/6-5/27/82)
1	20.0	19.0	18.0	20.1	20.8	19.0	20.9	18.9
2	20.0	20.5	17.8	19.6	20.2	18.7	20.9	19.0
3	20.5	19.0	17.0	19.0	19.7	18.2	21.0	19.0
4	21.0	20.0	16.5	18.6	19.3	17.9	20.7	19.0
5	20.0	20.0	16.4	18.1	18.9	17.6	20.6	19.0
6	20.0	20.0	16.6	18.7	18.7	17.0	20.3	18.7
7	20.5	20.0	20.5	22.6	21.6	17.5	20.6	18.7
8	22.0	20.5	24.6	25.1	25.3	20.7	22.3	21.2
9	25.0	22.5	28.4	28.4	26.6	25.9	23.4	23.2
10	24.5	26.5	30.0	30.1	30.2	28.9	25.5	24.5
11	25.0	29.0	31.0	31.4	32.9	31.6	28.8	24.9
12	27.5	31.3	32.1	32.8	33.0	33.4	30.3	26.4
13	29.0	32.0	30.8	32.9	32.9	31.8	30.7	27.1
14	29.0	31.3	30.4	33.3	32.1	30.0	29.6	26.4
15	27.5	30.7	29.6	32.2	31.5	28.9	29.0	26.0
16	25.5	30.3	29.4	30.3	30.4	28.0	27.8	25.4
17	23.5	27.0	27.0	28.4	28.6	26.8	26.0	22.6
18	21.5	24.7	26.6	26.4	26.7	24.7	23.5	20.7
19	21.0	21.7	24.4	25.0	25.0	23.6	22.3	20.2
20	20.5	20.5	22.1	22.5	23.7	23.1	21.9	19.0
21	21.0	20.5	20.4	22.4	22.7	21.4	21.7	18.5
22	20.0	20.5	19.8	21.8	22.0	20.7	21.5	18.8
23	20.0	20.5	18.9	21.3	21.4	20.2	21.2	18.9
24	20.0	20.0	18.3	20.8	21.1	19.7	21.0	19.0

*All times are military.

Appendix 4. Mean hourly relative humidity monitored within the fumigation chamber.

Hour of Day*	Ozone Concentration (Dates of Fumigation)							
	1.0 ppm (4/28-4/30/81)	0.75 ppm (4/30-5/2/81)	0.50 ppm (6/9-6/17/81)	0.25 ppm (6/17-7/8/81)	0.50 ppm (7/17-8/7/81)	0.40 ppm (8/11-9/1/81)	0.30 ppm (9/26-10/16/81)	0.25 ppm (5/6-5/27/82)
1	55.5	56.0	66.3	75.3	74.2	79.2	62.3	62.5
2	55.0	55.5	66.4	76.5	75.0	80.0	63.1	62.7
3	55.5	56.0	67.3	76.8	76.1	80.4	63.4	62.7
4	53.5	56.5	67.9	77.6	76.1	80.7	63.8	63.0
5	54.0	57.0	68.8	78.3	76.4	81.2	63.8	62.3
6	55.0	57.0	68.4	77.1	76.4	81.4	64.3	62.0
7	53.5	55.0	64.8	71.9	72.2	80.7	64.2	61.2
8	50.5	54.0	58.1	66.5	66.4	73.6	62.7	60.5
9	45.0	49.0	52.6	60.3	60.0	62.8	61.2	58.1
10	45.0	47.5	51.5	58.6	57.6	57.9	57.4	58.1
11	44.5	43.3	49.5	56.1	55.7	55.9	54.5	59.0
12	45.0	42.3	48.4	54.3	55.6	55.6	53.6	58.0
13	44.5	42.3	48.9	55.2	55.8	58.6	53.5	55.8
14	44.5	42.3	47.6	55.1	56.4	60.2	53.8	55.4
15	46.5	43.3	49.3	56.2	57.8	61.7	53.0	56.1
16	48.5	42.0	50.8	57.8	57.8	62.7	53.1	56.5
17	50.0	46.0	52.0	58.8	59.1	64.8	54.3	58.0
18	51.5	46.3	50.6	60.9	61.6	66.7	56.0	60.5
19	51.5	49.0	54.8	63.7	65.1	70.8	57.4	60.2
20	54.0	53.0	63.3	68.8	68.3	73.8	59.2	61.5
21	54.0	53.5	63.3	71.1	70.4	75.5	59.8	61.3
22	55.5	54.0	64.3	73.0	72.1	76.3	61.1	61.6
23	55.5	54.5	65.8	73.4	72.5	77.7	61.6	61.8
24	55.0	55.5	65.4	74.3	73.4	78.5	62.2	62.2

*All times are military.

Appendix 5. Mean hourly and daily temperature (C) and relative humidity monitored within the fumigation chamber during the 0.5 ppm chlorophyll extraction fumigation (6/2 - 6/20/82).

Time of Day* (hourly means)	Temperature	Relative Humidity
1	16.3	68.6
2	16.2	68.2
3	16.0	68.3
4	16.1	67.6
5	16.6	67.1
6	16.1	66.6
7	16.9	67.4
8	19.5	64.5
9	21.6	81.6
10	22.1	71.6
11	23.8	64.1
12	24.4	61.7
13	25.8	59.2
14	25.4	57.4
15	24.1	59.3
16	25.0	58.7
17	23.2	60.7
18	21.3	63.6
19	19.4	65.7
20	17.9	67.1
21	17.1	68.2
22	16.9	68.6
23	16.4	68.8
24	16.2	68.9

*All times are military.

Appendix 5 (cont')

Days from Start of Fumigation (daily means)	Temperature	Relative Humidity
1	18.1	73.1
2	18.0	72.0
3	20.7	65.4
4	21.5	58.7
5	19.3	55.4
6	21.1	61.8
7	20.8	57.8
8	23.1	59.7
9	21.0	62.4
10	20.0	68.5
11	21.1	72.8
12	20.3	71.0
13	18.9	64.7
14	16.8	73.2
15	21.7	66.3
16	18.8	72.5
17	17.2	68.3
18	20.9	64.0

Appendix 6. Average hourly and daily ozone concentrations monitored at two locations along the Colorado Front Range.

Time of Day*	Location (Dates of Monitoring)	
	Rist Canyon (9/9-9/22/81)	Conifer (10/25-11/7/81)
1	3.2	5.3
2	3.3	5.4
3	3.2	5.4
4	3.1	5.5
5	3.2	5.6
6	3.2	5.3
7	3.1	5.2
8	3.2	5.4
9	3.8	5.7
10	4.5	6.3
11	4.4	6.4
12	4.9	6.4
13	5.1	6.7
14	5.2	6.9
15	5.5	6.8
16	5.7	6.5
17	5.5	5.4
18	4.7	5.1
19	3.9	4.9
20	3.5	4.9
21	3.5	5.1
22	3.2	5.2
23	3.3	5.4
24	3.3	5.3

*All times are military.

APPENDIX 7

Fumigation Hints

1. The ozone generator utilized in the study had too large a capacity for the concentrations required in the research.

2. Cooling water to the generator must be maintained at a constant flow rate. This was accomplished by suspending a tank above the generator, maintaining a constant water level in the tank and throttling the flow with a needle valve on the tank outlet.

3. Ozone concentrations in the chamber can be controlled in three ways: 1) adjusting the rheostat on the ozone generator; 2) varying the speed of the chamber inlet blower; and/or 3) varying the reconstituted air flow rate into the ozone generator. Satisfactory results were obtained by incorporating all three methods. Fine adjustments were best made by utilization of a two-way needle valve varying the air flow rate into the ozone generator.

4. Although teflon film is the recommended material when building fumigation chambers, good results were obtained in this study by using inexpensive 8 mil polyvinyl chloride film purchased from the Livingstone Coating Corp., P.O. Box 8282, Charlotte, NC 28208 (phone 704-392-2323). Gluing was accomplished by using PVC cement and applied using a hypodermic syringe with the point filed flat.

APPENDIX 8

Indicator Plant Growth and Propogation

1. Quaking aspen (*Populus tremuloides* Michx.)--Three inch root sections were cut from aspen trees growing in the Arapahoe-Roosevelt National Forest. Root section ends were dipped in wax, placed horizontally in vermiculite (½ inch from the top) and placed into a mist bed until suckers developed. When suckers were 3 inches high they were cut off at the base with a sterile razor blade, dipped in Rootone and planted in vermiculite. Seedlings were watered as needed until a sufficient root system had developed (approx. 3-4 weeks), and then planted in 3.5 inch square plastic pots in a 4:1:1 (v/v) soil : vermiculite : perlite mix.

2. Plains cottonwood (*Populus sargentii* Dode.)--Plants were grown from seed collected in Fort Collins. Seeds were placed ½ inch deep in a 4:1:1 (v/v) soil : vermiculite : perlite mix in 3.5 inch square plastic pots and watered daily until germination, then watered as needed.

3. Narrowleaf cottonwood (*Populus angustifolia* James), gooseberry (*Ribes inerme* Rydb.) and willow (*Salix exigua* Nutt. and/or *Salix lucida* Muhl.)--Three inch shoot sections cut from plants growing in the Arapahoe-Roosevelt National Forest were dipped in Rootone, placed vertically in vermiculite (1 inch deep) and maintained in a

mist bed until a sufficient root system had developed (3-4 weeks). Plants were then planted in 3.5 inch square plastic pots in a 4:1:1 (v/v) soil : vermiculite : perlite mix and watered as needed.

4. Mullein (*Verbascum thapsus* L.)--Plants growing in the Arapahoe-Roosevelt National Forest were dug up whole and placed in 6 inch circular metal pots and watered as needed.